0013-7227/97/$03.00/0
Endocrinology

Vol. 138, No. 6
Printed in U.S.A.

Regulation of Iodothyronine Deiodinase Activity as Studied in Thyroidectomized Rats Infused with Thyroxine or Triiodothyronine*

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ABSTRACT

To provide new insights into the *in vivo* regulation of iodothyronine deiodinases in the different tissues of the rat, we have evaluated the effects on these enzymatic activities of T_4 or T_3 infusions into thyroidectomized rats.

Thyroidectomized rats were infused with placebo, T_4, or T_3. Placebo-infused intact rats served as euthyroid controls. Plasma and samples of cerebral cortex, brown adipose tissue, pituitary, liver, and lung were obtained after 12–13 days of infusion. Plasma TSH, plasma and tissue T_4 and T_3, and iodothyronine deiodinase activities were determined.

Type II 5′-deiodinase (DII) was increased in cortex, brown adipose tissue, and pituitary from animals infused with placebo. DII activity returned to normal only with T_4 infusion, remaining elevated in the animals infused with T_3 alone despite normal tissue T_3 concentrations. Cortex type III 5-deiodinase was only increased when hyperthyroidism was induced by infusion of T_3. Liver type I 5′-deiodinase (DI) paralleled the changes in plasma and tissue T_3 regardless of whether T_4 or T_3 was infused. On the contrary, the increase in lung DI, proportional to the increases in plasma and tissue T_3, was higher when T_4 was infused. As a rule, the tissues with DII presented a tighter homeostasis in their T_3 concentrations than the tissues with DI.

In conclusion, the regulation of iodothyronine deiodinases depends on the hormone infused into the thyroidectomized animals and on the tissue in which the deiodinase is studied, demonstrating the existence of tissue-specific regulation of its thyroid hormone concentrations. (*Endocrinology* **138:** 2559–2568, 1997)

THE METABOLISM of thyroid hormones is mediated by several enzymatic reactions, including conjugation, deamination, oxidative decarboxylation, and deiodination, which is the predominant mechanism (1). Deiodination of T_4 at the 5′-position of the phenolic ring can be considered a bioactivation, as the affinity of the nuclear thyroid hormone receptor for the resulting T_3 is 10- to 20-fold the affinity for T_4 (1–4). On the contrary, deiodination of T_4 at the 5-position of the tyrosyl ring results in an inactivation, as the resulting rT_3 has very low affinity for nuclear thyroid hormone receptors. Thus, deiodination is a key step in the regulation of thyroid hormone action, as approximately 80% of the T_4 secreted by the thyroid gland is deiodinated (5) either into the most active thyroid hormone, T_3, or into an inactive iodothyronine, rT_3. Deiodination is also involved in the main route of degradation of iodothyronines (*i.e.* inner ring deiodination of T_3) and plays a crucial role in the catabolism of sulfate conjugates of T_4 and T_3 in the liver (6). Thus, deiodination must not be considered an isolated event in thyroid hormone metabolism, but, rather, a highly regulated step that is coordinated with other metabolic reactions in several tissues.

Three major patterns of deiodination have been identified based on the activity measurements of tissue homogenates (7), which, according to affinity labeling studies, correspond to three separate enzymes (8–10), termed types I, II, and III iodothyronine deiodinases (DI, DII, and DIII). The genes responsible for these enzymes in several species have recently been cloned (11–15). Although the overall similarity of these genes is relatively low, there are three highly conserved limited regions, including a TGA codon that codes for selenocystein, which is essential for catalytic activity (15, 16).

DI can catalyze 5′-deiodination of nonsulfated iodothyronine, such as T_4 and rT_3, or the 5-deiodination of sulfated conjugates of T_4 or T_3 (T_4S and T_3S) (6, 17), and is extremely sensitive to inhibition by 6-propyl-2-thiouracil (PTU) and aurothioglucose (2). DI activity is found in liver, kidney, lung, pituitary gland, and thyroid (18, 19). Its affinity is higher for rT_3, T_4S, and T_3S than for T_4, and its role in liver, lung, and kidney seems to be the 5′-deiodination of rT_3 and the 5-deiodination of iodothyronine sulfates (20), whereas in the human and rat thyroid it catalyzes the 5′-deiodination of T_4 to T_3 (2) under the influence of TSH stimulation (21).

DII is present in the central nervous system, brown adipose tissue (BAT), anterior pituitary, and placenta (22–25). This enzyme is relatively insensitive to PTU, and its affinity is higher for T_4 than for rT_3. Brain DII has a primordial role in ensuring the adequate intracellular concentration of T_3 in

Received November 4, 1996.

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* Presented in part at the 76th Annual Meeting of The Endocrine Society, Anaheim, CA, June 1994, and the 22nd Annual Meeting of the European Thyroid Association, Vienna, Austria, September 1994. This work was supported by the Fondo de Investigaciones de la Seguridad Social (Proyecto 92/0888, BAE 93/5168 and 94/5082) and Henning Berlin (Berlin, Germany).

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the brain during critical periods of development in fetal and neonatal rats (26–28). In a recent study, we have also shown that increased DII activity in hypothyroid adult rats is able to normalize brain T_3 concentrations when T_4 is infused at very low doses that are insufficient to normalize even plasma T_4 and T_3 concentrations (29), providing a sensitive mechanism for maintaining brain T_3 homeostasis (27, 30). BAT DII significantly contributes to maintain T_3 concentrations in that tissue (31, 32), explaining the important role of T_3 in the full thermogenic response to cold exposure (33). Finally, pituitary DII contributes significantly to the T_3 that reaches the nuclear receptor, explaining how pituitary function can be modulated by circulating T_4 as well as by circulating T_3 (34).

DIII is resistant to PTU and aurothioglucose, and its affinity is higher for T_3 than for T_4 (2), catalyzing their conversion to 3′,3-T_2 and rT_3, respectively. This enzyme is present in brain, skin, placenta, and fetal tissues of the rat (35–37). This distribution suggests that DIII may have protective properties against high thyroid hormone concentrations during fetal development (2).

The regulation of deiodinase activity involves both pre- and posttranslational mechanisms and occurs in a tissue- and enzyme-specific manner (2). Although several factors, such as cold, fasting, cytokines, GH, retinoids, and drugs, influence deiodinase activity (16, 38), the main regulator is thyroid status. Hypothyroidism results in an increased activity of DII and thyroid DI and a decreased activity of liver and kidney DI and DIII. Opposite changes are noted in hyperthyroidism (1). It is well known that T_3 stimulates DI messenger RNA and activity in nearly all tissues studied (11, 39–42), and that thyroid hormones exert rapid inhibitory effects on DII (30).

Most previous pioneering studies have explored the regulation of the different iodothyronine deiodinases under conditions of severe hypo- or hyperthyroidism as well as their responses to injections of T_4 or T_3. This methodology does not permit exploration of the relationships between enzyme activities and concentrations of iodothyronines in serum or different tissues, as the time courses of possible changes in T_4 or T_3 concentrations and in the activities of the different deiodinases might differ both within the same tissue and among tissues. In the present study this problem was obviated by administering T_4 or T_3 by constant infusion, so that the different tissues would be in a steady state situation with regard to both the concentration of iodothyronines and the activities of the enzymes. As will be seen, the results provided new insights into the *in vivo* regulation of iodothyronine deiodinases in various tissues of the rat, and their consequences for the homeostasis of tissue thyroid hormone concentrations.

Materials and Methods

Experimental design

Young female Wistar rats, 120–150 g BW, were surgically thyroidectomized and received 100 μCi ^{131}I, ip, 1 week later. After 28 days, rats with complete body weight stasis were divided into groups of six rats each, and osmotic minipumps (Alzet, model 2ML2, Alza Corp., Palo Alto, CA) were implanted under the dorsal skin of the animals. In three separate experiments, the rats were infused with placebo solution, T_4 (Exp A: 1.0, 2.0, 3.0, 4.0, and 8.0 μg/100 g BW·day; Exp B: 0.2, 0.4, 0.6, 0.8, and 1.6 μg/100 g BW·day), or T_3 (Exp C: 0.25, 0.50, 0.75, 1.00, and 2.00 μg/100 g BW·day). One group of seven nonthyroidectomized rats, matched for sex and age and infused with placebo, served as the control euthyroid group in each experiment.

After 12–13 days of infusion the rats were bled and perfused, as previously described (29), after being slightly anesthetized with ether. Samples of plasma, cerebral cortex, pituitary, BAT, liver, lung, and other tissues were obtained for the present and other studies. Samples were immediately frozen on dry ice and stored at −20 C until analyzed, with the exception of aliquots of cortex, pituitary, BAT, liver, and lung that were stored at −80 C for measurement of iodothyronine deiodinase activity. The thyroid hormone tissue and plasma concentrations as well as some preliminary data for deiodinase activities of T_4-infused rats were previously reported (29).

Determinations

T_4 and T_3 were measured in whole plasma and in tissues after extraction and purification of the iodothyronines by specific and highly sensitive RIAs, as detailed previously (29, 43).

TSH was measured in plasma using immunoreactants kindly provided by the Rat Pituitary Agency of the NIDDK, NIH (Bethesda, MD), as described previously (29, 43). Results are expressed in weight equivalents of the NIDDK rTSH RP-3 preparation.

DII activity was assayed in cortex and BAT (31) using 2 nM T_4, 1 μM T_3, and 20 mM dithiothreitol (DTT) in the presence of 1 mM PTU, and the reaction time was 60 min. DI activity was assayed in liver, pituitary, and lung homogenates as previously described (26), using 400 nM rT_3 and 2 mM DTT for liver, and 2 nM rT_3 and 20 mM DTT for pituitary and lung, in 100 mM potassium phosphate buffer (pH 7.0). The reaction time was 10 min for liver and 60 min for pituitary and lung. Pituitary DII activity was evaluated using 2 nM rT_3 and 20 mM DTT in the presence of 1 mM PTU. Before each assay [^{125}I]rT_3 or [^{125}I]T_4 was purified by paper electrophoresis to separate the contaminating iodide. The $^{125}I^-$ released was separated by ion exchange chromatography on Dowex 50W-X2 (BioRad, Richmond, CA) columns equilibrated in 10% acetic acid. The production of equal amounts of iodide and 3′,3-diiodothyronine was checked in some assays. The protein content was determined as previously described (26, 27, 31) after precipitation of the homogenates with 10% trichloroacetic acid to avoid interference from DTT in the colorimetric reaction.

DIII activity was measured in cortex homogenates (29), incubating 20–50 μg protein/100 μl in 100 mM potassium phosphate buffer (pH 7.4), 1 mM EDTA with approximately 50,000 cpm of inner ring-labeled [^{125}I]T_3 (3,5-[^{125}I]T_3), 50 nM T_3, 20 mM DTT, and 1 mM PTU for 60 min at 37 C. Radioiodide release was measured as described above. When necessary, 3,5-[^{125}I]T_3 was purified just before the assay using disposable Sep-Pak C_{18} cartridges (Waters Associates, Milford, MA).

Drugs and reagents

T_4, T_3, 3,5-diiodothyronine, PTU, and DTT were obtained from Sigma Chemical Co. (St. Louis, MO). rT_3 and 3′,3-diiodothyronine were obtained from Henning Berlin (Berlin, Germany).

High specific activity [^{131}I]T_4, [^{125}I]T_3, [^{125}I]T_4, and [^{125}I]rT_3 (3000 μCi/μg) were synthesized in our laboratory and used for highly sensitive T_4 and T_3 RIAs, as recovery tracers for plasma and tissues extractions, and as substrates for 5′-deiodinases. The inner ring-labeled 3,5-[^{125}I]T_3 (80 μCi/μg), used as substrate for DIII, was kindly provided by Drs. R. Thoma and H. Rökos from Henning Berlin.

Statistical analysis

One-way ANOVA and the protected least significant difference test for multiple comparisons were used after validation of the homogeneity of variances by the Bartlett-Box *F* test. Square root or logarithmic transformations usually ensured homogeneity of variances when this was not found with the raw data. Results are expressed as the mean ± SEM. $P < 0.05$ was considered significant in all comparisons. To compare the results from the three experiments and to compare the degree of change observed in the plasma with those in various tissues, iodothyronine deiodinase activity and T_4, T_3, and TSH concentrations in samples from each thyroidectomized rat and from each thyroidectomized rat receiving different T_4 or T_3 doses are expressed in the figures as percentages of the

TABLE 1. Mean (±SEM) values of iodothyronine deiodinase activities (DI, DII, and DIII, in femtomoles of I^- per h/mg protein, except for liver DI, which is in picomoles of I^- per min/mg protein) in control intact rats (C), thyroidectomized rats (Tx) infused with placebo, and Tx rats infused with T_4 or T_3

Exp A												
Group:	C	Tx		Tx		Tx		Tx		Tx		Tx
T_4 dose:[a]	0	0		1.0		2.0		3.0		4.0		8.0
Cortex DII	9.6 ± 0.8	87.8 ± 7.7	▲[b]	8.3 ± 0.5	=	7.6 ± 0.4	=	9.3 ± 1.8	=	6.3 ± 0.4	▼	6.1 ± 0.3	⇳
Cortex DIII	2022 ± 473	290 ± 41	▼	861 ± 159	=	1405 ± 429	=	1619 ± 242	=	2499 ± 486	=	1270 ± 177	=
BAT DII	17 ± 2	265 ± 34	▲	45 ± 8	▲	17 ± 2	=	13 ± 1	=	16 ± 1	=	24 ± 3	▲
Liver DI	26 ± 2	5 ± 1	▼	20 ± 4	=	41 ± 3	▲	46 ± 5	▲	60 ± 8	▲	94 ± 9	▲
Lung DI	204 ± 16	68 ± 3	▼	209 ± 47	=	218 ± 10	=	292 ± 12	=	291 ± 32	▲	422 ± 72	▲
Exp B												
Group:	C	Tx		Tx		Tx		Tx		Tx		Tx
T_4 dose:[a]	0	0		0.2		0.4		0.6		0.8		1.6
Cortex DII	33 ± 2	209 ± 10	▲	123 ± 11	▲	77 ± 19	▲	27 ± 3	=	35 ± 6	=	22 ± 1	=
Cortex DIII	1354 ± 194	1692 ± 603	=	2346 ± 768	=	1742 ± 679	=	2249 ± 677	=	739 ± 161	=	3438 ± 1452	=
BAT DII	320 ± 30	1106 ± 76	▲	692 ± 36	▲	381 ± 75	=	508 ± 86	=	856 ± 38	▲	147 ± 24	=
Pituitary DII	350 ± 9	2466 ± 117	▲	2092 ± 205	▲	1615 ± 250	▲	1486 ± 175	▲	854 ± 72	▲	408 ± 16	=
Pituitary DI	3085 ± 72	1216 ± 62	▼	1505 ± 138	▼	1972 ± 134	▼	1721 ± 78	▼	1904 ± 121	▼	2486 ± 95	⇳
Liver DI	52 ± 4	16 ± 2	▼	22 ± 2	▼	18 ± 2	▼	28 ± 2	▼	31 ± 2	▼	52 ± 4	=
Lung DI	557 ± 17	144 ± 9	▼	166 ± 16	▼	183 ± 9	▼	245 ± 39	▼	283 ± 31	▼	359 ± 19	=
Exp C												
Group:	C	Tx		Tx		Tx		Tx		Tx		Tx
T_3 dose:[a]	0	0		0.25		0.50		0.75		1.00		2.00
Cortex DII	75 ± 8	403 ± 11	▲	537 ± 10	▲	575 ± 29	▲	617 ± 30	▲	454 ± 4	▲	360 ± 31	▲
Cortex DIII	1487 ± 94	1111 ± 367	=	1299 ± 291	=	1754 ± 167	=	1883 ± 185	=	2588 ± 488	▲	3241 ± 522	▲
BAT DII	508 ± 86	1429 ± 105	▲	1035 ± 44	▲	1184 ± 35	▲	901 ± 55	▲	1303 ± 85	▲	817 ± 79	▲
Pituitary DII	657 ± 12	2953 ± 140	▲	2243 ± 227	▲	1327 ± 38	▲	987 ± 80	▲	939 ± 67	▲	1040 ± 43	▲
Pituitary DI	3940 ± 109	1822 ± 235	▼	3107 ± 368	=	4438 ± 237	=	6082 ± 402	▲	7836 ± 315	▲	7587 ± 628	▲
Liver DI	16 ± 1	12 ± 1	▼	19 ± 1	⇳	32 ± 1	▲	45 ± 2	▲	52 ± 2	▲	126 ± 8	▲
Lung DI	551 ± 37	184 ± 13	▼	515 ± 40	=	774 ± 19	▲	1081 ± 87	▲	923 ± 96	▲	1314 ± 59	▲

Cortex, cerebral cortex; BAT, brown adipose tissue.

[a] The doses are in micrograms per 100 g BW/day.

[b] The symbols represent the comparison of the mean values from T_4- or T_3-infused rats with those from control rats infused with placebo: = indicates that there were no statistically significant differences; and ▼ identifies low levels compared to controls, with at least $P < 0.05$; ▲ identifies elevated levels compared to controls, with at least $P < 0.05$. An open arrow (⇳ or ⇳) indicates that, although statistically significant, the change with respect to controls was relatively small, within ±30% of the mean of the control group.

mean value corresponding to the group of intact controls, which was taken as 100%.

To assess the relationships between tissue and plasma T_3 and T_4 concentrations and deiodinase activity, individual paired data, expressed as percentages of the mean value of the group of intact controls, as stated above, were adjusted to several curves (linear, logarithmic, inverse, compound, power, sigmoidal, growth, and exponential). The curve fit with the highest coefficient of determination (r^2), degrees of freedom, and F statistics was selected for each variable. When T_3 alone was used as replacement therapy for hypothyroidism, the plasma T_4 concentration was not included in the curve fitting because its values were below the limit of detection of our assay in all groups of thyroidectomized rats (plasma T_4 resulted in a constant rather than a variable). Curve fittings with $r^2 < 0.50$, despite statistical significance, are usually not considered of biological significance, as in such a case less than 50% of the changes in the dependent variables are explained by related changes in the independent variable. Statistical analyses were performed with the SPSS for Macintosh versions 4.0 and 6.1.1 (SPSS, Chicago, IL).

Results

The iodothyronine deiodinase activities, as a function of the dose of T_4 or T_3 infused, are described in Table 1 and represented in Figs. 1-4. The plasma and tissue thyroid hormone concentrations[1] are represented in Figs. 1–4. The results of the curve fitting of iodothyronine deiodinase activities as a function of plasma and tissue thyroid hormone concentrations are represented in Figs. 5–7, and their curve fitting as a function of plasma TSH is described in Table 2.

Plasma concentrations of thyroid hormones

In the thyroidectomized rats infused with placebo the plasma concentrations of T_4 and T_3 were very low, and the concentrations of TSH were very high (Fig. 1). In the rats infused with T_4, normal plasma T_4 concentrations were reached in the groups infused with 0.6–1.0 μg/100 g BW·day, normal plasma T_3 concentrations were obtained in the groups infused with 1.0 and 1.6 μg/100 g BW·day, and both increased above normal at higher T_4 doses (Fig. 1). Plasma TSH concentrations were normal only in the group infused

[1] To permit comparisons between the different experiments, the thyroid hormone concentrations are expressed in the figures as percentages of the mean values of the control group. The absolute values of plasma and tissue thyroid hormone concentrations and plasma TSH concentrations in T_4-infused rats were described in detail previously (29). The absolute values of plasma and tissue thyroid hormone concentrations and plasma TSH concentrations in T_3-infused rats can be derived from the data points shown in Figs. 1–4, where the 100% values corresponding to the placebo-infused control group of Exp C were: plasma T_4, 40 ± 2 ng/ml; plasma T_3, 0.79 ± 0.08 ng/ml; plasma TSH, 0.89 ± 0.12 ng/ml; cerebral cortex T_4, 1.73 ± 0.25 ng/g; cerebral cortex T_3, 2.67 ± 0.14 ng/g; BAT T_4, 3.73 ± 0.24 ng/g; BAT T_3, 3.40 ± 0.17 ng/g; pituitary T_4, 67 ± 9 pg/gland; pituitary T_3, 94 ± 6 pg/gland; liver T_4, 22.74 ± 2.77 ng/g; liver T_3, 3.67 ± 0.22 ng/g; lung T_4, 7.51 ± 0.18 ng/g; and lung T_3, 1.24 ± 0.01 ng/g.

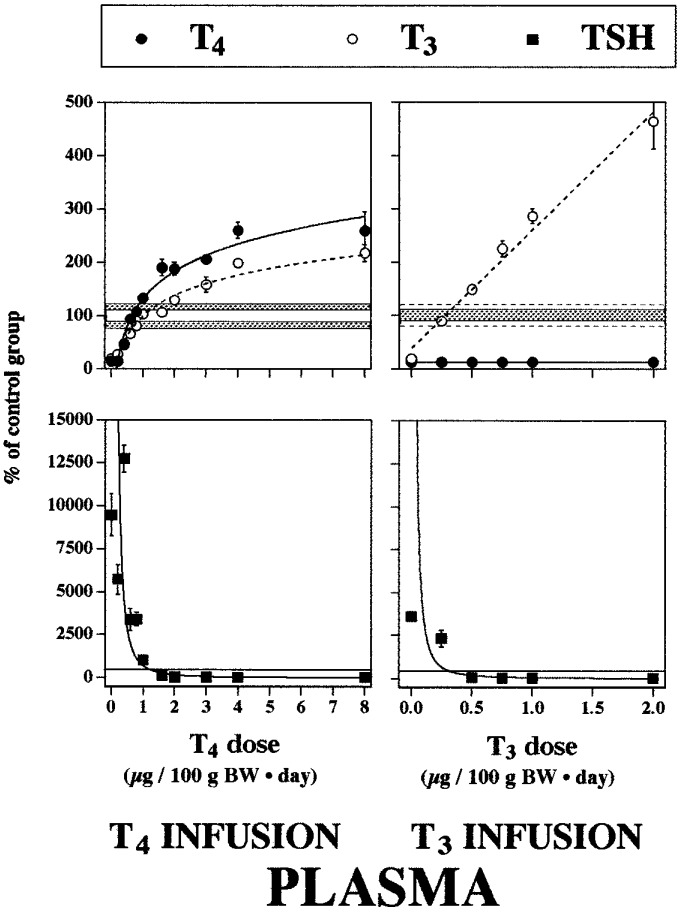

FIG. 1. The changes in plasma T_4 and T_3 concentrations (*upper panels, full and empty circles*, respectively) and in plasma TSH (*lower panels, full squares*) of thyroidectomized rats infused with placebo, T_4 alone (*left panels*), or T_3 alone (*right panels*) are represented as a function of the dose of T_4 or T_3. Values shown are the mean ± SEM and are expressed as percentages of the mean value in control intact animals (see Footnote 1). The absence of vertical lines for the SEMs is due to their being within the dimension of the symbol. The *areas enclosed by horizontal lines* represent the 95% confidence intervals of the plasma T_4 (*full lines, dotted area*) and T_3 (*dotted lines, white area*) concentrations of intact control rats. The *horizontal line in the lower panels* resulted from the area representing the 95% confidence interval of plasma TSH concentrations of the control group, which appears as a line because of the reduction in the size of the figure. The *two left panels* showing the plasma T_4, T_3, and TSH concentrations in T_4-infused animals are taken from the report by Escobar-Morreale *et al.* (29) and are reproduced from *The Journal of Clinical Investigation* 96:2828–2838, 1995, by copyright permission of the American Society for Clinical Investigation and with permission of the authors.

with 1.6 μg/100 g BW·day; plasma TSH levels were elevated in the groups infused with lower T_4 doses and decreased in the groups infused with higher T_4 doses (Fig. 1).

Plasma T_4 concentrations were below the limit of detection of the assay in all groups of thyroidectomized rats infused with T_3 (Fig. 1). Plasma T_3 was already normal in the group infused with 0.25 μg/100 g BW·day and elevated in the groups infused with higher T_3 doses (Fig. 1). Finally, the plasma TSH concentration was elevated in the group infused with 0.25 μg/100 g BW·day and low in the groups infused with higher T_3 doses (Fig. 1).

Cerebral cortex DII

Cerebral cortex DII activity was markedly elevated in thyroidectomized rats infused with placebo (Table 1 and Fig. 2). When T_4 was infused into thyroidectomized rats, cortex DII activity showed a progressive decrease, reaching normal activities with T_4 doses between 0.6–3.0 μg/100 g BW·day, whereas the groups infused with the higher T_4 doses (4.0 and 8.0 μg/100 g BW·day) showed decreased DII activity with respect to controls (Table 1 and Fig. 2). Cerebral cortex T_3 concentrations reached normal levels with T_4 doses from 0.4–8.0 μg/100 g BW·day, with the exception of a slight increase in the group infused with 1.6 μg/100 g BW·day (Fig. 2). On the contrary, cortex T_4 concentrations were low in the groups infused with 0.2–0.8 μg/100 g BW·day and high in the groups infused with 1.0–8.0 μg/100 g BW·day (Fig. 2).

In contrast to the result of infusion of T_4 alone, when T_3 was infused into thyroidectomized rats, cortex DII activity remained elevated, as none of the doses of T_3 infused was able to normalize its activity. Moreover, DII activity was further increased, compared to the activities found in animals infused with placebo, with T_3 doses of 0.25, 0.50, and 0.75 μg/100 g BW·day (Table 1 and Fig. 2). Cerebral cortex T_3 concentrations reached normal levels in the group infused with 0.75 and 1.00 μg/100 g BW·day and were elevated with the higher T_3 dose (Fig. 2). As expected, cerebral cortex T_4 concentrations were low in all groups infused with T_3 (Fig. 2).

DII activity in the cerebral cortex of rats infused with T_4 was related to both plasma and cortex T_4 and T_3 (Fig. 5). In the rats infused with T_3, DII activity was only related to cortex T_4 and plasma T_3, but the curve fittings were not strong enough ($r^2 < 0.50$) to ensure biological significance (Fig. 5).

Cerebral cortex DIII

DIII activity in cerebral cortex showed inconsistent results in the groups infused with placebo, as this activity was reduced with respect to that in the control group only in Exp A, but remained normal in Exp B and C (Table 1). In the groups infused with T_4, cerebral cortex DIII activity was not different from the control value, and the small changes present between the groups infused with different T_4 doses were not related to the changes in cortex T_4 or T_3 levels (Table 1).

On the contrary, in the animals infused with T_3 alone, DIII activity increased with the higher T_3 doses and higher cortex T_3 concentrations (Table 1 and Fig. 2). In this experiment, the changes in DIII activity were weakly related to the changes in cortex and plasma T_3 (compound curve fitting: $r^2 = 0.43$; $P < 0.01$; power curve fitting: $r^2 = 0.37$; $P < 0.01$).

BAT DII

BAT DII activity was markedly elevated in thyroidectomized rats infused with placebo (Table 1 and Fig. 2). When T_4 was infused, DII activity showed an irregular pattern of response, showing a tendency to normalization with the higher T_4 doses (Table 1 and Fig. 2). BAT showed an acceptable homeostasis in its T_3 concentrations, which were normal

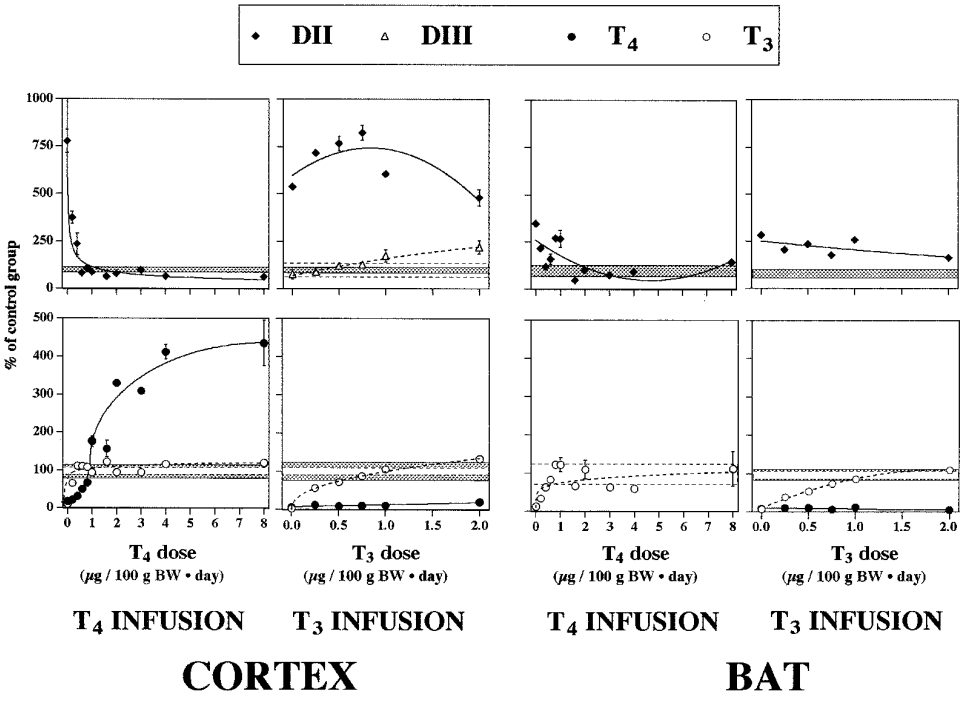

FIG. 2. The changes in DII (*full diamonds*) and DIII (*open triangles*) activities and in tissue concentrations of T_4 (*full circles*) and T_3 (*empty circles*) in cerebral cortex and BAT of thyroidectomized rats infused with placebo, T_4 alone, or T_3 alone are shown as function of the dose of T_4 (*left panels*) or T_3 (*right panels*). As nonsystematic changes were found in the cerebral cortex DIII activity of rats infused with T_4 alone, their plots have been removed from the figure. The tissue T_4 concentrations of BAT in the animals infused with T_4 have been removed from the figure for the reasons outlined in the text. The expression of data and the meaning of *dotted and white areas* are explained in Fig. 1. The panels showing the T_4 and T_3 concentrations in the cerebral cortex and BAT of T_4-infused animals are taken from the report by Escobar-Morreale *et al.* (29) and are reproduced from *The Journal of Clinical Investigation* 96:2828–2838, 1995, by copyright permission of the American Society for Clinical Investigation and with permission of the authors.

in all groups infused with T_4, with the exception of low levels in the groups infused with 0.2, 0.4, and 1.6 μg/100 g BW·day (Fig. 2). As occurred with BAT DII activity, the changes in BAT T_4 concentrations were highly irregular. These T_4 values might be questioned because of the proximity of the interscapular BAT pads to the site where the osmotic pump is implanted and from which it is only separated by an easily punctured, thin layer of connective tissue. Although care was taken at autopsy to avoid contact between the BAT pads and fluid surrounding the T_4-containing pumps, some external contamination with T_4 might have occurred; for this reason the data have not been shown.

BAT DII activity decreased when T_3 was infused into thyroidectomized rats, but its activity did not reach normal values at any of the T_3 doses tested (Table 1 and Fig. 2), although BAT T_3 concentrations were normal in the groups infused with 0.75 μg/100 g BW·day or more (Fig. 2). As expected, T_4 concentrations were very low in all groups of thyroidectomized rats infused with T_3 (Fig. 2).

BAT DII activity in thyroidectomized rats receiving T_4 alone showed sigmoidal relationships to plasma T_3 and T_4 (Fig. 5). Curve fitting of BAT DII activity against tissue and plasma T_4 and T_3 concentrations disclosed only weak relationships in rats infused with T_3 (Fig. 5).

Pituitary DII and DI

Pituitary DII activity was markedly elevated, and DI activity was markedly decreased, in thyroidectomized rats infused with placebo (Table 1 and Fig. 3).

The effects of T_4 infusion on pituitary 5′-deiodinase activities were evaluated only in Exp B, as, unfortunately, no aliquots of pituitary homogenate were saved before extraction in Exp A. Pituitary DII activity showed a progressive decrease with the increasing T_4 doses infused, reaching normal values with the dose of 1.6 μg/100 g BW·day (Table 1 and Fig. 3). Pituitary DI activity showed the opposite change, increasing with the increasing T_4 doses, but remained slightly decreased (81% of the mean value of the control group) with respect to the control value with the higher dose of 1.6 μg/100 g BW·day (Table 1 and Fig. 3). Pituitary T_4 concentrations were low in the group infused with 0.2 μg/100 g BW·day; normal in the groups infused with 0.4, 0.6, 1.0, and 2.0 μg/100 g BW·day; and elevated in the groups infused with 0.8, 1.6, and 3.0–8.0 μg/100 g BW·day (Fig. 3). Pituitary T_3 concentrations reached normal levels in the groups infused with T_4 doses ranging from 1.0–3.0 μg/100 g BW·day and were elevated in those given higher T_4 doses (Fig. 3).

When the animals were infused with T_3, the changes in pituitary DII activity resembled those in BAT. Although there was a progressive decrease in DII activity, it remained elevated in the group infused with the higher T_3 dose (Table 1 and Fig. 3). On the contrary, pituitary DI activity reached normal levels in the groups infused with 0.25 and 0.50 μg/100 g BW·day and was elevated with higher T_3 doses (Table 1 and Fig. 3). Pituitary T_3 reached normal concentrations in the groups infused with 0.50 and 0.75 μg/100 g BW·day and was elevated with higher T_3 doses (Fig. 3). As expected, pituitary T_4 concentrations were low in all groups infused with T_3 (Fig. 3).

Finally, both pituitary DI and DII activities in thyroidectomized rats given T_4 alone were related to pituitary and plasma T_4 and T_3 (Fig. 6), and the best fit was obtained for both deiodinases with pituitary T_4. On the contrary, when the animals were infused with T_3 alone, pituitary DI and DII activities were only related to plasma and pituitary T_3 (Fig. 6).

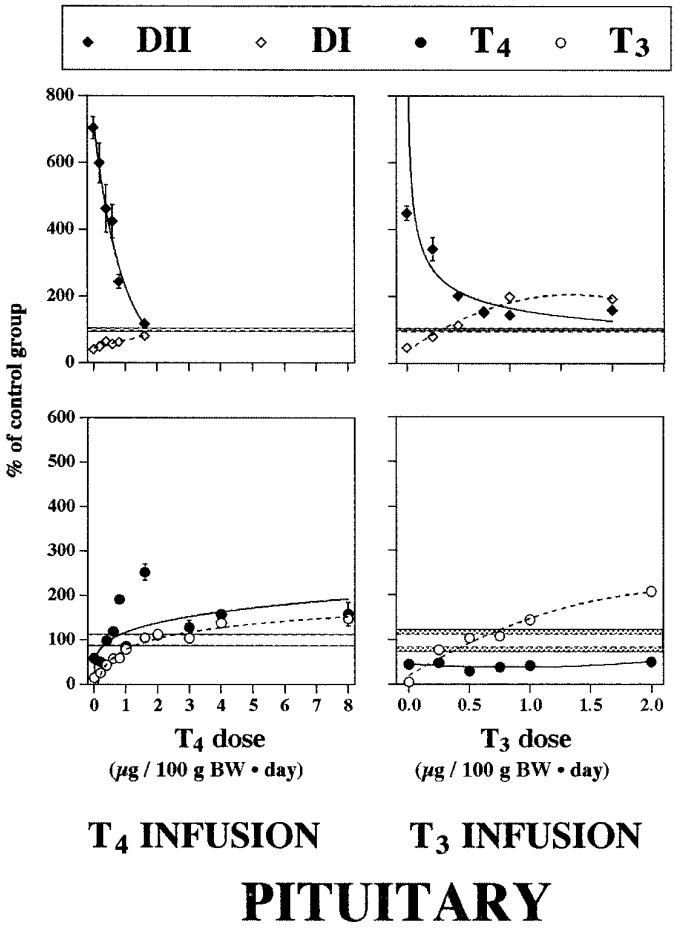

FIG. 3. The changes in DII (*full diamonds*) and DI (*open diamonds*) activities and in tissue concentrations of T_4 (*full circles*) and T_3 (*empty circles*) in the pituitary of thyroidectomized rats infused with placebo, T_4 alone, or T_3 alone are shown as function of the dose of T_4 (*left panels*) or T_3 (*right panels*). The expression of data and the meaning of *dotted and white areas* are explained in Fig. 1.

Liver DI

Liver DI activity was markedly decreased in thyroidectomized rats infused with placebo (Table 1 and Fig. 4). In the rats infused with T_4, liver DI activity reached normal levels with 1.0 and 1.6 μg/100 g BW·day and was elevated with higher T_4 doses (Table 1 and Fig. 4). The changes in liver DI activity paralleled closely those in plasma T_3, whereas the concordance with liver T_3 and T_4 was not complete (Table 1 and Fig. 4). Liver DI activity in rats infused with T_4 was related to plasma T_4 and T_3 and to liver T_3 and T_4 (Fig. 7).

Liver DI activity was increased in all groups infused with T_3, showing some concordance with liver T_3 (Table 1 and Fig. 4). Liver DI activity was related to plasma and liver T_3 and, surprisingly, to liver T_4 (Fig. 7), although the latter concentrations were extremely low (Fig. 4).

Lung DI

Lung DI activity was markedly decreased in thyroidectomized rats infused with placebo (Table 1 and Fig. 4). In the rats infused with T_4, lung DI activity reached a normal value with 1.0–3.0 μg/100 g BW·day (the dispersion in the lung DI activity of the control group was high) and was elevated with higher T_4 doses (Table 1 and Fig. 4). The changes in lung DI activity paralleled those in lung T_3 (Fig. 4) and were mainly related to plasma and lung T_3 and, to a lesser degree, plasma and lung T_4 (Fig. 7).

When T_3 was infused into thyroidectomized rats, lung DI activity was normal with the dose of 0.25 μg/100 g BW·day and elevated with higher T_3 doses (Table 1 and Fig. 4). Similar changes were observed in lung T_3 concentrations, whereas lung T_4 levels were very low (Fig. 4). Lung DI activity was related to plasma and lung T_3 (Fig. 7).

Relationships between plasma TSH concentration and iodothyronine deiodinase activities

Cerebral cortex and BAT DII activity showed weak or nonsignificant relationships with plasma TSH concentrations regardless of whether T_4 or T_3 was infused into thyroidectomized rats (Table 2). On the contrary, the changes in pituitary DII activity and in pituitary, liver, and lung DI activities were related to the changes in plasma TSH concentrations (Table 2). Pituitary DII activity was directly related to TSH, as expected from the fact that both increase in response to hypothyroidism, whereas DI activity in pituitary, liver, and lung, was inversely related to the change in plasma TSH, reflecting the expected opposite response to the change in thyroid function (Table 2). Finally, the change in cerebral cortex DIII in the animals infused with T_3 alone showed a weak, but significant, relationship with plasma TSH (Table 2).

Discussion

Thyroidal status is the main regulator of iodothyronine deiodinase activity. DII activity increases in response to hypothyroidism (30) and decreases in response to hyperthyroidism. On the contrary, DI activity decreases in hypothyroid situations and increases in hyperthyroidism, with the exception of the thyroidal enzyme, which is under the control of plasma TSH (21). Our results show that the degree of response of each enzyme to thyroidal status is different depending on the tissue studied and the hormone infused.

Type II 5′-deiodinase and type III 5-deiodinase

As expected, DII activity is increased in cerebral cortex, BAT, and pituitary in thyroidectomized animals infused with placebo. In the three tissues studied, DII activity decreases in response to T_4 infusion, reaching normal levels after tissue T_3 euthyroidism is reached. However, when T_3 is infused, DII activity does not normalize even in the presence of normal or high tissue T_3 concentrations. In contrast to BAT and pituitary DII, which tended to decrease with the increase in T_3 concentrations, cerebral cortex DII activity actually increases in this situation. Recent results from our group have shown a similar T_3-induced increase in DII activity in fetal BAT (44). Present results point to T_4, but not T_3, as the main down-regulator of cerebral cortex and BAT DII activity (27, 29). Moreover, the tight tissue homeostasis of the T_3 concentration in the cerebral cortex and BAT is only maintained when T_4 is supplied to the thyroidectomized animals,

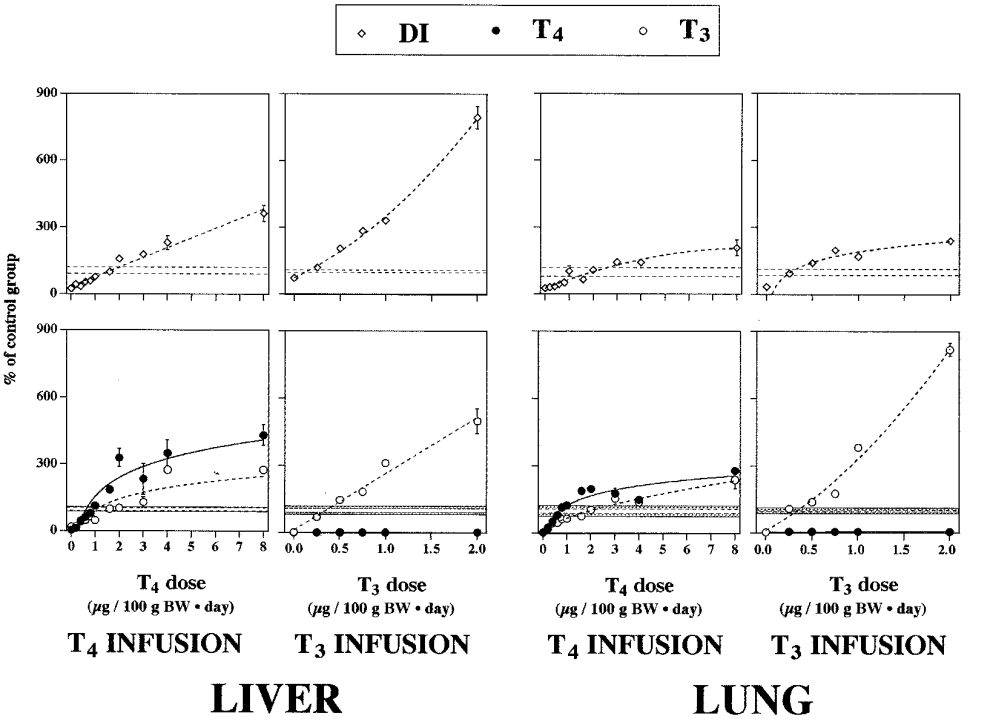

FIG. 4. The changes in DI activity (*open diamonds*) and tissue concentrations of T_4 (*full circles*) and T_3 (*empty circles*) in liver and lung of thyroidectomized rats infused with placebo, T_4 alone, or T_3 alone are shown as function of the dose of T_4 (*left panels*) or T_3 (*right panels*). The expression of data and the meaning of *dotted and white areas* are explained in Fig. 1. The panels showing the T_4 and T_3 concentrations in the liver and lung of T_4-infused animals are taken from the report by Escobar-Morreale *et al.* (29) and are reproduced from *The Journal of Clinical Investigation* 96:2828–2838, 1995, by copyright permission of the American Society for Clinical Investigation, and with permission of the authors.

TABLE 2. Results of the curve fitting between iodothyroine deiodinase activity and plasma TSH in thyroidectomized rats infused with T_4 or T_3 alone

Deiodinase[a]	Plasma TSH-T_4 infusion		Plasma TSH-T_3 infusion	
	Fit[b]	r^2	Fit	r^2
Cerebral cortex DII	Power	0.38	No significant fitting was found	
Cerebral cortex DIII	No significant fitting was found		Compound	0.32
BAT DII	Power	0.30	Linear	0.20
Pituitary DII	Power	0.72	Linear	0.93
Pituitary DI	Logarithmic	0.54	Power	0.80
Liver DI	Power	0.70	Sigmoid	0.78
Lung DI	Power	0.75	Inverse	0.78

Before curve fitting, raw data were transformed into percentages of the mean value of the control group. The 95% confidence interval for the normal plasma TSH concentrations were 77–123% of the mean control values (TSH, 1.02 ng/ml).

[a] DI, Type I 5′-deiodinase; DII, type II 5′-deiodinase; DIII, type III 5-deiodinase; BAT, brown adipose tissue.

[b] The curve with the best fitting (defined by the highest r^2 and F values) was selected. Unless otherwise stated, all fittings were significant ($P < 0.05$).

further suggesting that DII activity and T_4 are intimately related, with the former using the latter as substrate to provide T_3, especially in hypothyroid situations. Finally, although a stimulatory effect of TSH on DII expression has been described in astrocytes (45), our data have failed to confirm this hypothesis, as the changes in cortex DII activity in rats infused with T_3 did not shown any relationship with changes in plasma TSH.

Pituitary DII activity showed a different pattern of response to T_4 infusion compared to DII in the cortex and BAT. Pituitary DII needed elevated pituitary T_4 concentrations and normal pituitary T_3 concentrations to normalize its activity. This result might be related to the presence of DI activity in pituitary, as the T_3 produced from T_4 by pituitary DI might be exported into the circulation instead of contributing to local T_3 concentrations. In fact, a similar physiological role has been suggested for DI activity in other tissues, such as liver and kidney (2). In contrast to the cerebral cortex and BAT enzymes, pituitary DII activity showed a strong relationship with pituitary and plasma T_3 during T_3 infusion, a result that suggests that at least to some extent in the pituitary, DII activity is sensitive to the inhibitory effects of T_3. This conclusion is also supported by others using single iodothyronine injections *in vivo* (46). Unfortunately, we were not able to measure pituitary iodothyronine deiodinase activities in Exp A, and thus, we do not have data regarding the pattern of response of these activities in T_4-induced hyperthyroidism.

When hyperthyroidism was induced by infusion of very high doses of T_4 or T_3, cerebral cortex DII activity only showed a mild decrease with T_4 infusion and remained elevated when T_3 alone was infused. BAT DII activity did not decrease with respect to the control value and, in fact, was slightly elevated with the highest T_4 dose infused. In this situation, cerebral cortex and BAT T_3 concentrations were normal or only mildly elevated (cerebral cortex T_3 in

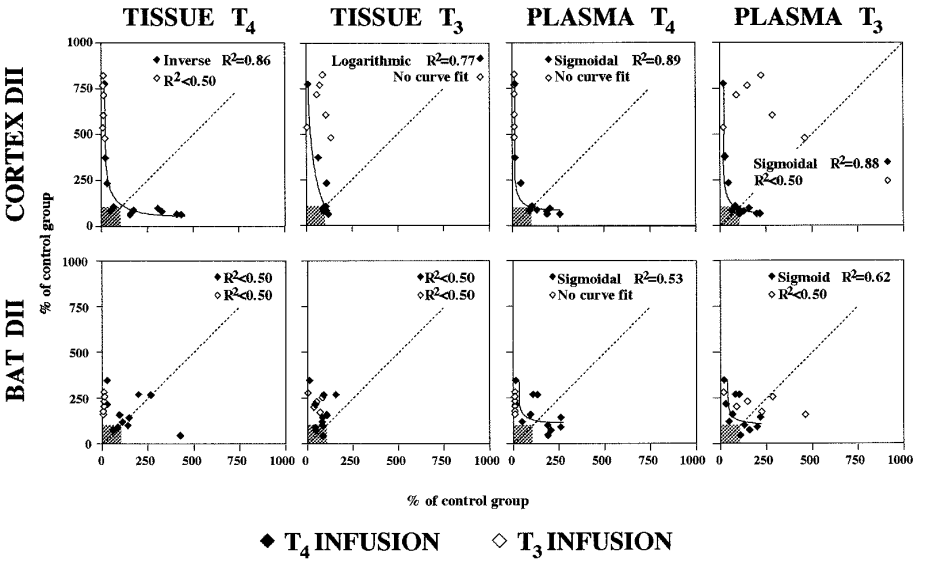

FIG. 5. Best-fit curves obtained for DII in cerebral cortex and BAT of thyroidectomized rats infused with T_4 alone (*full symbols*) or T_3 alone (*open symbols*) as a function of tissue and plasma T_4 or T_3 concentrations. The values are expressed as a percentage of the mean value in the control group. The *dotted square inset* separates the values below and above those in control intact rats. The *thin diagonal line* corresponds to the relationship that would be found if the changes in deiodinase activity were accompanied by changes of equal intensity in T_4 or T_3 concentrations. R^2 is the coefficient of determination. Values below 0.5 seldom have biological significance.

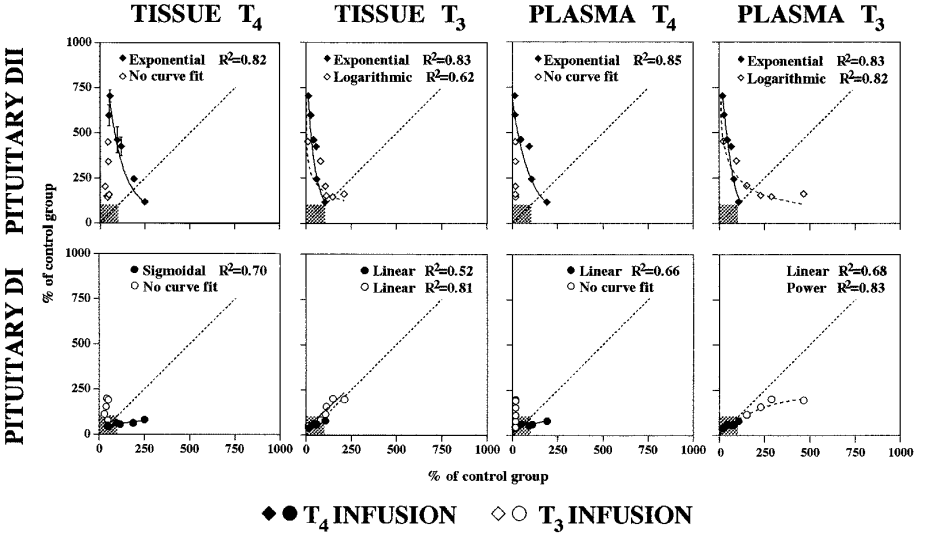

FIG. 6. Best-fit curves obtained for DI (*circles*) and DII (*diamonds*) activities in the pituitary of thyroidectomized rats infused with T_4 alone (*full symbols*) or T_3 alone (*open symbols*) as a function of tissue and plasma T_4 or T_3 concentrations. The values are expressed as a percentage of the mean value in the control group. The meaning of *dotted areas and lines* is the same as indicated in Fig. 5. R^2 is the coefficient of determination. Values below 0.5 seldom have biological significance.

animals infused with T_3), pointing to mechanisms other than inhibition of DII as those responsible for the protection of brain and BAT against hyperthyroidism. DIII is present in the central nervous system and catalyzes the inactivation of T_4 into rT_3 and that of T_3 into T_2. Although some researchers have found a decrease in brain DIII activity in hypothyroidism and an increase in hyperthyroidism, with the latter having protective properties against elevated thyroid hormone concentrations (47), we have not been able to confirm these results. Cerebral cortex DIII showed low activities in thyroidectomized rats infused with placebo only in Exp A, not in Exp B and C. Conversely, elevated DIII activity was only found in the groups infused with the higher T_3 doses in Exp C, but not in animals in which hyperthyroidism was induced by the infusion of T_4. The latter result might be related to a high efficiency of DIII in the degradation of T_4 and T_3, thus maintaining normal or near-normal tissue T_3 concentrations with minor increases in DIII activity. On the other hand, if T_3 homeostasis is achieved through other regulatory mechanisms, the changes in tissue T_3 might not be enough to induce changes in DIII activity. Other mechanisms not implicating iodothyronine deiodinases, such as reduction in the transthyretin-mediated T_4 transport at the choroid plexus (48) or a decreased T_3 permeability of the blood-brain barrier, might be involved in protection of the cerebral cortex against hyperthyroidism, but do not explain the homeostasis of BAT T_3 or pituitary T_3.

DI

As expected, DI activity was decreased during hypothyroidism and increased during hyperthyroidism in pituitary, liver, and lung. However, the magnitude of the changes in response to thyroidal status was not the same in all of these tissues, pointing to tissue-specific factors regulating deiodi-

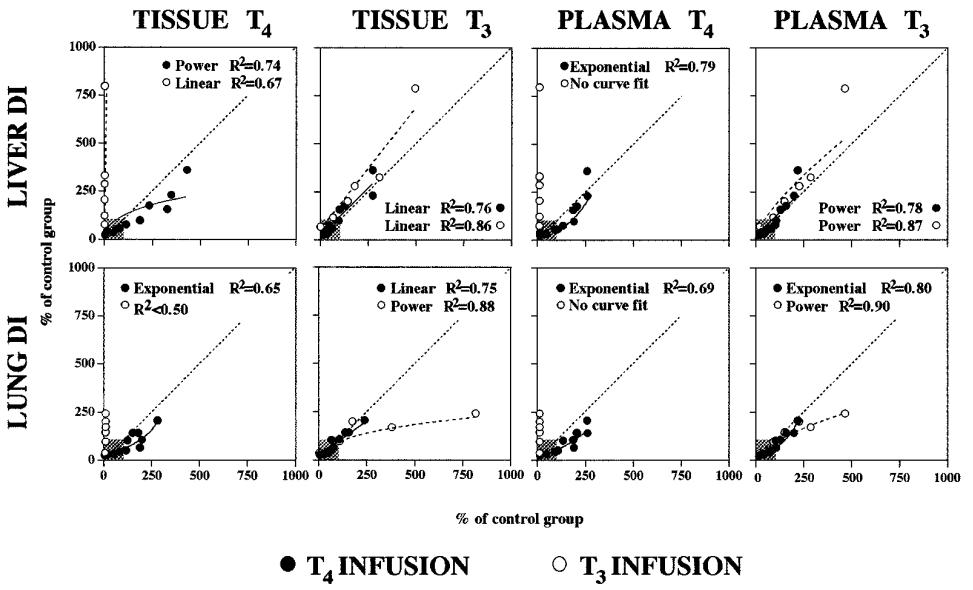

FIG. 7. Best-fit curves obtained for DI activity in liver and lung of thyroidectomized rats infused with T_4 alone (*full symbols*) or T_3 alone (*open symbols*) as a function of tissue and plasma T_4 or T_3 concentrations. The values are expressed as a percentage of the mean value in the control group. The meaning of *dotted areas and lines* is indicated in Fig. 5. R^2 is the coefficient of determination. Values below 0.5 seldom have biological significance.

nase activity. Moreover, the relationships of iodothyronine deiodinase activity with plasma and tissue thyroid hormones were different depending on which iodothyronine, T_4 or T_3, was infused.

Pituitary DI activity increases nearly in parallel with pituitary T_3 when either T_4 or T_3 is infused. This finding suggests a regulatory role of pituitary T_3 on pituitary DI activity, in agreement with studies performed in pituitary cells *in vitro* (49). With infusion of increasing doses of T_3, plasma T_3 reached an approximately 5-fold increase with respect to the control value, whereas pituitary T_3 and DI activity only showed 2-fold increases. On the contrary, when T_4 alone was infused, changes in plasma T_3, pituitary T_3, and pituitary DI activity were similar. This result is in agreement with previous studies in which most of pituitary T_3 is derived from local conversion of T_4 to T_3 (23, 50). When T_4 is absent, the only source of pituitary T_3 is the circulating T_3, and supraphysiological plasma T_3 concentrations must be reached to normalize pituitary T_3.

Liver DI activity changed in parallel with plasma and tissue T_3 regardless of whether T_4 or T_3 was used as replacement therapy. The highest increases in liver DI activities were reached with the highest T_4 or T_3 dose and were approximately 1.5-fold the simultaneous increases in plasma and liver T_3. On the contrary, lung DI activity was differentially affected by changes in thyroid hormone concentrations depending on whether T_4 or T_3 was infused. During T_4 infusion, lung DI activity changed in parallel with plasma T_4 and T_3 and tissue T_3. On the contrary, when T_3 was infused, the increase in lung DI activity was approximately a third of the increase in plasma and lung T_3. This might result from a stimulatory effect of T_4 or the lung T_3 generated by local conversion from T_4 on lung DI activity and a lack of effect of lung T_3 derived directly from plasma. Other possibilities include an enhanced sulfation of thyroid hormones in lung during T_4 infusion, which, in turn, might stimulate lung DI activity, or a saturation of the stimulatory effect of T_3 on DI activity when very high levels of lung T_3 are reached during T_3 infusion.

Summary

The results of the present study demonstrate that the *in vivo* regulation of iodothyronine deiodinases in the rat is different depending on the tissue studied. Especially important is the fact that our experimental design has permitted us to study the mechanisms that regulate tissue iodothyronine concentrations under steady state conditions resulting in variable degrees of hypo- or hyperthyroidism. This experimental approach might mimic the physiological and pathological abnormalities of thyroid function better than previous studies performed with single or intermittent injections of T_4 or T_3.

The present results further support the existence of tissue-specific mechanisms that regulate thyroid hormone concentrations in target tissues (29). None of the single doses of T_4 or T_3 tested has been able to normalize each iodothyronine deiodinase simultaneously in the tissues in which it was present or even to simultaneously normalize DI and DII in tissues with both activities (*i.e.* the pituitary). Thus, the pattern of regulation by thyroid hormones of the iodothyronine deiodinases depends on the tissue studied, the iodothyronine infused, its dose, and, possibly, the route of administration, explaining the frequent discrepancies found in the literature regarding this issue. The same occurs with the T_4 and T_3 concentrations in plasma and tissues (29). On the contrary, tissue concentrations of T_4 and T_3 and iodothyronine deiodinase activities in the tissues studied here reach normal values simultaneously when a combination of 0.9 μg T_4 and 0.15 μg T_3/100 g BW·day is infused, as previously reported (43).

According to our present results, tissues with DII activity show a high degree of homeostasis of their T_3 concentrations. The increase in DII activity explains the efficiency of cerebral cortex, BAT, and pituitary in the normalization of tissue T_3 in situations of hypothyroidism, but does not explain the protection against elevated circulating thyroid hormone levels shown by these tissues. The possible role of cortex DIII in this protection has been only partially supported by our data. Finally, as opposed

to tissues with DII activity, tissues in which DI activity is preferentially expressed maintain thyroid hormone concentrations similar to those in plasma. This finding might be explained by two nonexclusive hypotheses for such tissues: their thyroid hormone concentrations are in equilibrium with circulating levels and/or the T_3 generated in these tissues is the main source of circulating T_3.

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